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EFFECT OF HEMORRHAGE ON COMPLEMENT OF BLOOD

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The effects of hemorrhage on the cellular elements of the blood have been fairly well established. According to Drinker,[1] within 10 minutes after hemorrhage, the leukocyte count begins to rise, and reaches its height within a few hours. Following a single large hemorrhage it may remain elevated for a week. Next comes an increase in platelets, which begins to be manifest during the first 24 hours, and reaches a maximum within the first few days. The young red forms are slower to appear; and the red count is made up much more slowly than either of the others, depending on the size and duration of the hemorrhages. The hemoglobin content is the last to return to normal. The anemia following chronic conditions of loss of blood according to Lee and Minot[2] shows less tendency to marked leukocytosis, less tendency to marked increase in immature forms, but aplasia of the blood forming organs never results from hemorrhage. As pointed out by Robertson and Bock,[3] the fluid content of the blood is not fully made up until the third day; and hence the red count may continue to fall until that time, due to gradual dilution, unless fluids are forced on the patient.

On the other hand, the relation between these changes and complementing power of the blood have not been studied. Coca[4] states that the regeneration of complement after hemorrhage, when the bulk of the lost blood is replaced with inactivated serum and washed corpuscles, is more rapid than can be accounted for, on the grounds of the addition of complement to the blood by the lymph, the latter being supposed to have the same titer as the normal blood. It, therefore, seemed to us desirable to ascertain the changes induced in the complement of the blood by profound and multiple hemorrhages and subsequent blood regeneration; and what, if any, were the relations of this change to changes in the cellular elements.

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[1] Oxford Medicine, 1920, 2, p. 509.

[2] Nelson Loose Leaf Medicine, 1920, 4, p. 17.

[3] Jour. Exper. Med., 1919, 29, p. 155.

[4] Ztschr. f. Immunitätsf., 1914, 20, p. 622.

Guinea-pigs were used on account of the known stability of the complement titer in this animal. The normal titer was established in all cases, except in the last series, by 3 preliminary titrations and 2 blood counts. The ear veins were found satisfactory for occasional bleeding, but when repeated withdrawals of blood in amounts of 1.5 c c or more, coupled with additional punctures for blood counts, were necessary, heart puncture was found to be the only practical method.

The counting of platelets was best accomplished in a solution of 3.8% sodium citrate, plus 0.2% formaldehyd and enough brilliant cresyl blue 0.1% to stain the platelets a deep blue. The solution was found to become hemolytic (?) unless made fresh every few days, and was always filtered immediately before using. The methods of Pratt,[5] Wright and Kinnicutt,[6] and Buckman and Hallisey[7] were tried and discarded. The method used allowed the counting of red cells and platelets in the same preparation, the red count being checked from time to time with Hayem's solution. The procedure was as follows: A red-counting pipet was first filled with diluting fluid to the 0.5 mark; blood was drawn rapidly until the top of the column of fluids touched the 1 mark, when the pipet was again plunged into the diluting fluid. The resulting dilution was 1:200.

The serum for titration was obtained, as a rule, from 1.5 cc of blood and was diluted 1:20, in which dilution a variation of 2 tubes is not to be considered significant when a fresh hemolytic series had to be prepared each day. When, however, an interval of only a few hours allowed the use of the same hemolytic series, with identical pipet, etc., the variation of a single tube is to be noted. An excess of amboceptor (4 units) was used.

During the entire experiment the animals were fed on fresh vegetables, but were given no fluids to drink. To avoid the danger of a complicating infection the animals after the first pair were not tagged by piercing the ear.

The first series covered a period of 30 days up to the death of animal B, although the observations were somewhat irregular. Animal A was used again later, and observations on this animal covered 74 days and a total of 6 more or less severe hemorrhages. From repeated small hemorrhages the animals reached a stage of mild chronic anemia, as shown by the immature red forms and the slight reaction of leukocytes and platelets following the last bleeding. The differential leukocyte count showed a progressive shift toward the mononuclear elements at the expense of the polymorphonuclear neutrophils. No constant or significant variation in complement was made out.

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[5] Jour. Am. Med. Assn., 1905, 45, p. 1919; 1906, 46, p. 1092.

[6] Ibid., 1911, 56, p. 1457.

[7] Ibid., 1921, 76, p. 427.

The second series reached a more profound anemia, the relation of the red count to complement titer being shown in chart I. Except for a slight drop in complement in the specimen taken 3 hours after the first hemorrhage, which was regained the next day, there were no changes in titer attributable to hemorrhage. Animal C, which was pregnant when the experiment was undertaken, showed a somewhat irregular but generally weaker titer up to parturition; it is interesting to note that the titer promptly returned to what appears subsequently to be the normal level, and did so in spite of 3 successive hemorrhages. The behavior of leukocytes and platelets, both as to numerical and differential count, was similar to that of the preceding series, except for the appearance of a few myelocytes.

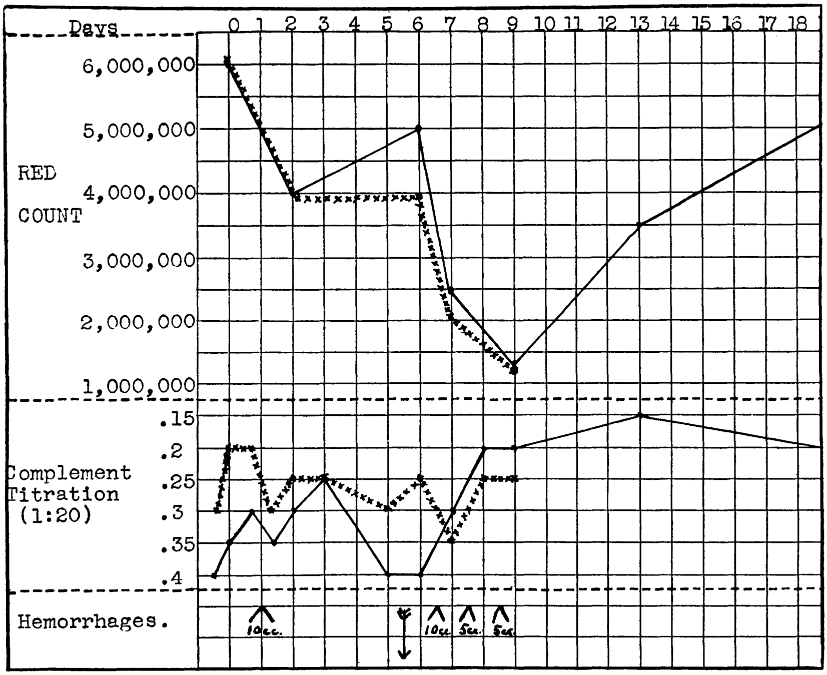

Chart 1.—Relation of red count to complement titer in the second series of animals. The curve of animal C is indicated by the x x x line, that of animal D by the continuous line.

Animals E and F of series three showed characteristic cellular reactions following a single severe hemorrhage, but no significant changes in complement titer. In this experiment blood was tested one hour after hemorrhage. In contrast to the previous finding, the titer was unaltered.

Apparently no appreciable or constant variation in the titer of the blood can be made out 24 hours after a hemorrhage even of great severity. Neither the subsequent dilution nor any event in the course of regeneration of the cellular elements affects the titer. The reaction of red cells and platelets in a typical animal is shown in chart 2, in which dilution is seen not to be complete until the third day after a severe hemorrhage. The leukocyte counts are incomplete and the reactions insufficient to show a characteristic curve. That repeated hemorrhages reducing the red count to about one-sixth of normal and

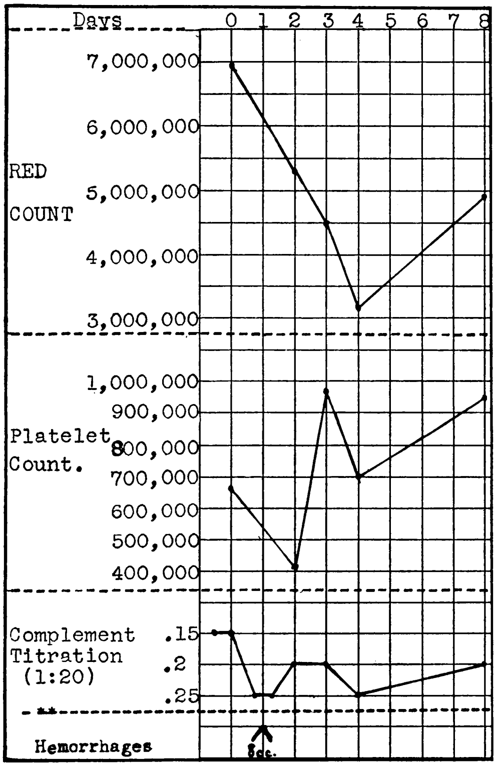

Chart 2.—Reaction of red cells and platelets in a typical animal.

prolonging the period of anemia have also no effect on the level of complement present in the blood, is also to be deduced from the several animals on which this was tried. The rise in titer following parturition is interesting but does not come within the scope of this paper.

The observation that the complementing power showed a distinct drop 3 hours after hemorrhage led us to add the following experiment:

Eight guinea-pigs, of which the red and platelet counts had been determined, where bled from 10 to 11 c c according to their weight, which ranged around 600 gm. The normal complement figure for each animal was obtained from this blood. Following this counts were made and blood titrated from 1 or more animals at intervals of 1 hour, so that in the end each animal had been used twice, besides the original hemorrhage. The greatest care was taken to make the results exactly comparable.

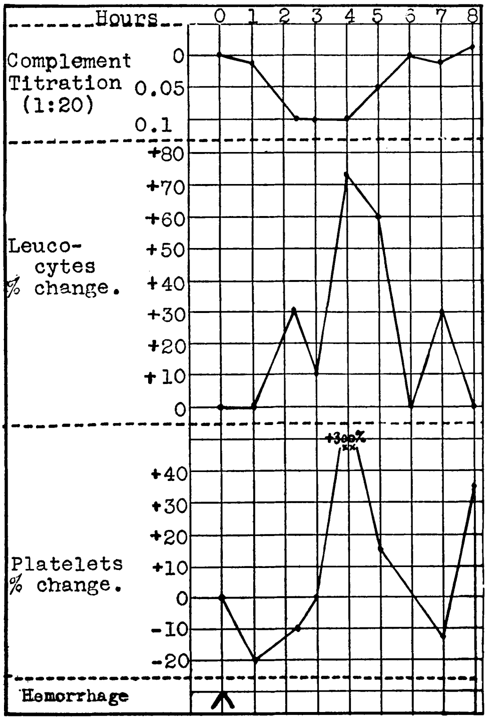

Chart 3.—Results in third experiment.

The result is best given in chart 3 in which the observations are combined and averaged. The leukocyte count will be seen to begin to rise after 1 hour, while the platelet count shows an initial drop. As dilution is considered as beginning at once, this should mean an immediate production of leukocytes, which reaches a maximum within a few hours and then slows down. The platelets, on the other hand, begin their regeneration more slowly and show greater individual variation. Previous experience would indicate that the count does not remain above normal until after 24 hours, although production is undoubtedly taking place, as shown by the fluctuations in count, resulting in practically offsetting the rate of dilution.

The curve of complement shows the beginning of a drop after 1 hour (1 out of 4 animals), but after 2½ hours there is a weakening of 2 tubes. This weakening was constant for all animals until the fifth hour when the titer began to increase, and by the sixth hour the complementing power seems to have returned almost constantly to normal. In reading these titrations the results were rendered difficult by the fact that in addition to the weakening, the end-point of complete hemolysis was rendered less distinct, and several tubes might show almost complete hemolysis before one could be called clear. This was a contrast to the shart end-points of the normal titrations.

The origin of complement, then, is not in the cells of circulating blood. There appears to be no relation between the curve of complement regeneration and the reactions of the various cellular elements. That the tissue fluids first called on to make up the lost plasma are poor in complement would seem to be the reason for the initial drop. There is probably a reservoir or factory of complement, as yet unknown, whose response is fairly prompt and very accurate, since an overproduction comparable to that found in leukocytes and platelets is not seen. In using this terminology, it must not be supposed that anything is assumed regarding the nature of this substance, for we are not in a position to say that it is elaborated after any particular manner.

SUMMARY

Complement has a regular curve of decrease and regeneration following a single severe hemorrhage.

It is unaffected by subsequent dilution of the blood by the body fluids.

The curve does not run parallel to that of any of the cellular elements circulating in the blood following hemorrhage, nor is there reason to believe that any of these cells is a factor in the regeneration of complement.

The initial decrease is due to dilution of the blood, which decrease is soon overcome and restored to normal by an influx of complement from some, as yet unknown, source.

Repeated hemorrhages with extreme degrees of anemia and subsequent recovery do not alter the level of complement.